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1
Carboxypeptidase H is an Autoantigen of the ICA and is Expressed on the Cell Surface of Islet Cells. Alvin C. Powers, Sarah Bowen, Sandra West, Nashville, TN.

The islet molecules targeted by the islet cell autoantibodies (ICA) of Type I diabetes are incompletely characterized. To identify the protein targets of the ICA, we have created a human islet cell λ GT 11 cDNA library from human insulinomas and screened this library with ICA sera. We have identified 8 clones which react with only an ICA serum and not with normal sera. One of the clones reacts with two/six ICA sera and the cDNA insert has been analyzed by DNA sequencing and RNA analysis. This 1.4 kb cDNA has greater than 80% homology with the rat carboxypeptidase H (CPH) cDNA sequence and likely encodes human CPH. RNA analysis with the labeled cDNA detects a 2.5 kb mRNA species in poly-A RNA from human insulinomas and RIN 1046-38, Beta TC-1, and alpha TC-6 cell lines. CPH mRNA is also found in poly-A RNA from human kidney, adrenal, and testes, but not from liver, duodenum, spleen, or fibroblasts.

CPH is a proinsulin processing enzyme within the beta cell secretory granule and exists as a membrane and soluble form. To determine if CPH is expressed on the cell surface and thus accessible to the immune system, viable Beta TC-1 cells were stained with an anti-CPH serum and cell surface fluorescence analyzed by flow cytometry. At least 25% of the Beta TC-1 cells were positive for cell surface staining for CPH when analyzed three hours after the addition of the anti-CPH serum. These results suggest that CPH is an autoantigen of the ICA and is expressed on the cell surface of islet cells.

3
Cloning and Expression of Islet Cell Autoantigens, D. RABIN,* S. PLEASIC, R. PALMER-CROCKER, P.M.M. RAE*, J. SHAPIRO, J. BARBOSA*, W. KNOWLES, C. ROWE and J. OLES, West Haven, CT

A DNA cloning approach was taken to define, purify, and characterize islet cell antigens that are recognized by Type I diabetic sera. Such antigens could be useful in diagnosis of pre-Type I diabetes, and could help provide markers for the study of autoimmune aspects of the disease.

A cDNA library was generated in bacteriophage λ -gt11 from human islet material (provided by P. Lacy and D. Scharp, St. Louis MO) and screened with sera from newly diagnosed diabetics. Plaques that were reactive with the diabetic sera were expressed in *E. coli* and immune precipitated with diabetic and normal sera.

Clone ICA512 was recognized by 16/32 (50%) diabetic sera and 0/20 (0%) normals by immunoprecipitation and 41/104 (39%) diabetic and 1/61 (1.6%) normals by ELISA reactivity.

Sequence analysis of ICA512 reveals partial homology to human LCA (CD45).

Preliminary results with two other antigens that show diabetic specificity will also be presented.

2
Utilization of a human λ gt11 islet library to identify novel autoantigens associated with Type I diabetes. M. PIETROPAOLO, L. CASTANO, E. RUSSO, *A. POWERS, O. BARNEA, GS.EISENBARTH. Boston, MA, *Nashville, TN.

We have in the past identified with sera from prediabetic relatives a clone producing carboxypeptidase H [termed DG-1 (codes for amino acid 199 to 335 of carboxypeptidase H)] in a rat cDNA library. Recently we have utilized a cDNA expression library (A. Permutt, St. Louis, MO) from human pancreas islets to screen for clones reacting with our rat carboxypeptidase H probe and novel autoantigens. Our rat islet probe hybridized with 23 human carboxypeptidase reactive clones after screening 25×10^5 plaques which are being sequenced. In addition utilizing antibodies from prediabetic relatives we have recently identified in this library what we believe is a novel islet antigen. This clone, termed PM-1, reacts with 2 out of 6 ICA positive relatives screened to date, whereas none of 10 control sera react. The labeled PM-1 insert detects a 2.0 Kb mRNA species in total RNA from a human insulinoma, a human islet carcinoid cell line (BON-1), and 3 rodent islet cell lines (RIN 1046-38, BTC-1, α TC-6). No hybridization was detected in total RNA from 3 human, non-islet cell lines (HepG2-hepatoma, HeLa-fibroblast, JEG-choriocarcinoma), suggesting that the PM-1 clone reacts with an islet protein expressed in all human and rat pancreatic islet cell lines and human insulinoma. Initial sequence shows a 252 bp open reading frame coding for 84 amino acids without significant homologies to known sequences in Gene Bank and containing two regions of dibasic aminoacids. In summary autoreactive molecules can readily be isolated from a human λ gt11 expression library and we believe will contribute to characterize the family of autoantigens of prediabetics and should facilitate identification of novel islet molecules.

4
Characterization Of The Antigen Recognized By The Islet-Specific T Cell Clone BDC-2.5, BARBARA BERGMAN and KATHRYN HASKINS, Denver, CO.

Disease transfer studies with the islet-specific T cell clone BDC-2.5 show it is able to accelerate the disease process in young, unirradiated nonobese diabetic (NOD) mice resulting in hyperglycemia by six weeks of age. BDC-2.5 was derived from a newly diabetic NOD mouse and is of the CD4 phenotype. In *in vitro* assays, BDC-2.5 proliferates and makes IL-2 in response to NOD antigen presenting cells and islet cell antigen isolated from a number of mouse strains. In addition, the mouse beta tumor cell lines β TC3 and NIT-1 can serve as sources of antigen. Islet cell membranes, as well as whole islet cells and islet cell lysates, can stimulate BDC-2.5, suggesting a cell surface antigen. We are currently investigating the ability of anti-islet cell antibodies to inhibit the proliferative response of BDC-2.5 to islet cell antigen. We have identified at least three antibody reagents that appear to react with the antigen recognized by the T cell clone.